

ROLE OF SHODHANA ON ANALYTICAL PARAMETERS OF *DATURA INNOXIA* MILL AND *DATURA METEL* LINN SEEDS

Patel Yogesh¹, Bhat Savitha D.², Acharya Rabinarayan^{3*}, B. K Ashok⁴, Shukla V.J⁵

¹M Pharm (Ayu.) scholar, PGT-SFC, Gujarat Ayurved University, Jamnagar, Gujarat, India

²PhD scholar, Department of Dravyaguna, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

³Reader in Department of Dravyaguna, IPGT & RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

⁴Senior Research Fellow, CCRAS, Pharmacology lab, IPGT&RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

⁵Head, Pharmaceutical chemistry lab, IPGT&RA, Gujarat Ayurved University, Jamnagar, Gujarat, India

Received: 02-10-2010; Revised: 20-10-2010; Accepted: 26-10-2010

ABSTRACT

Dhattura is a well known and frequently used drug in Ayurveda. Since its seeds are considered to be highly toxic, Ayurveda advocates specific procedures called *Shodhana* (purification procedures) before rendering it into a safe therapeutic drug. The present analytical study was carried out on seeds of two species of *Dhattura* namely *Datura innoxia* Mill. and *Datura metel* Linn. both before and after *Shodhana*. Parameters like physico-chemical analysis, pH, extractive values, test for various functional groups, thin layer chromatography and GC-MS studies with special reference to hyoscyamine and scopolamine alkaloids were carried out. The *Shodhana* process resulted in 70 to 90% reduction in hyoscyamine content, where as scopolamine content reduced almost to zero. The present study reflects the importance of *Shodhana* of poisonous plant drugs by means of which one can lessen the toxic constituents.

KEYWORDS: *Dhattura*, *Shodhana*, Physico chemical study, Analytical study, Hyoscyamine, Scopolamine.

***Corresponding Author**

Dr Rabinarayan Acharya,
Reader, Department of Dravyaguna,
I.P.G.T & R.A,
Gujarat Ayurved University,
Jamnagar,
Gujarat. 361008
Email: drnacharya@gmail.com

INTRODUCTION

In Ayurvedic pharmacopeia, *Dhattura* is a well known drug which comes under the *Upavisha varga* (Plants with poisonous effects) as described in various classical texts^{1,2}. In one of the classical lexicon of *Dravyaguna Raja Nighantu*, five varieties of *Dhattura* have been reported namely *Shweta*(White), *Krishna*(Black), *Peeta*(Yellow), *Rakta*(Red) and *Neela*(Blue), among which *Krishna* variety is said to be therapeutically more potent than others³.

Different species of *Dhattura* like *Datura innoxia* Mill, *Datura metel* Linn., *Datura stramonium* Linn., *Datura alba* Linn., etc. have been identified and many authors have correlated them with the above varieties⁴. However for therapeutic purposes *D. metel* and *D. innoxia* are commonly used.

For treating various ailments different parts of the *Dhattura* like leaves, seeds and roots are used. But the seeds are reported to be highly toxic which is evidenced by various animal studies⁵⁻⁸ also and hence seeds are mainly subjected for purification Ayurvedic classics have emphasized this fact by mentioning various methods of *Shodhana* (purification) with different medias like *Gomutra* (Cow's urine), *Godugdha* (Cow's milk), etc^{9,10}. Since a comparative analytical data on seeds of these two species before and after purification through different medias was not available, this study was undertaken to know the impact of *Shodhana* on their phyto-constituents.

MATERIALS AND METHODS

Mature seeds of *Datura innoxia* and *Datura metel* were collected from their natural source and authenticated by the help of pharmacognosist of IPGT & RA, Gujarat Ayurved University, Jamnagar. The seeds were shade dried and preserved in air tight glass container until further use. Both the samples of *Dhattura* seeds were subjected to *Shodhana* as per classical method mentioned in the text *Rasamruta*¹⁰. About 100 grams of *Datura innoxia* Mill. seeds were soaked in 400ml of freshly collected *Gomutra* and kept aside for twelve hours. Then the *Gomutra* was decanted and the seeds were washed in water. It was then subjected to *swedana* in *Godugdha* for three hours using *dolayantra* (a type of instrument used for boiling). The seeds were again washed in hot water and allowed to dry. The outer covering of the seeds (testa) was removed and stored. The same procedure was followed for seeds of *Datura metel* also.

The *Ashodhita* (Unpurified/Raw) and *Shodhita* (Purified) seed samples of both the species were subjected to analytical study. The drugs were coded as; Sample A: *D.metel* Linn. (*Ashodhita*), Sample B : *D.metel* Linn. (*Shodhita*), Sample C : *D.innoxia* Mill. (*Ashodhita*), Sample D: *D.innoxia* Mill. (*Shodhita*)

ANALYTICAL STUDY

Physico-chemical analysis namely loss on drying at 110°C, total ash, acid insoluble ash, water soluble extractive value, alcohol soluble extractive value and petroleum ether soluble extractives were carried out for seed samples. The test for various functional groups like alkaloids, flavanoids, glycosides, tannins, carbohydrates, tri-terpenoids etc were also carried out by using standard procedures¹¹. Quantitative estimation of total alkaloid content and total protein content was also carried out¹². To analyse the pH, 10% (w/v) aqueous solution of the sample was prepared and filtered. pH of the filtrate was noted in an Ellico's digital pH meter using combined glass electrode¹³. The methanolic extract of all the samples were subjected to TLC fingerprint analysis using silica Gel (GF 254) as stationary phase and methanol: liquor ammonia (100: 1.5) as solvent front. The plates were developed in iodine chamber and the R_f values were calculated^{14,15}. For GC-MS analysis, about five grams of coarse powder was moistened with eight millilitre of ammonium hydroxide and dried. Then the dried powder was extracted with 10ml of alcohol and partitioned with 20ml of ether. Thus extracted alkaloids were subjected for GC-MS analysis to estimate the percentage of Hyoscyamine and Scopolamine¹⁶.

RESULTS AND DISCUSSION

Dhattura occupies an important position in Ayurvedic therapeutics. Though seeds are considered to be more disruptive and have shown to produce deleterious effects, it is rendered therapeutically safe through *Shodhana* procedures. Hence the change in the phytochemical profile of the drug after *Shodhana* will be of utmost importance.

About 24.52% decrease in loss on drying, 47.68% and 130% increase in total ash value and acid insoluble ash value respectively was observed in seeds of *D.metel* after *Shodhana*. Whereas *Shodhita D.innoxia* seeds showed 44.20% increase in loss on drying, 10.75% decrease in total ash value and 130% increase in acid insoluble ash. The variation in the percentage of loss on drying by *D.innoxia* may be due to retaining of some moisture factor from the media like *Godugdha* used for *Shodhana*. Total ash content of crude drug is the inorganic residue remaining after incineration. The observed increase in the ash value of *D.metel* may be due to gaining of inorganic matter from the media. An apparent increase in acid insoluble ash was may be due to some acid insoluble particles like silica gained during the *Shodhana*. **Table 1** shows data related to different physicochemical parameters of the two *Dhattura* samples before and after *Shodhana*.

Water soluble and alcohol soluble extractive values were decreased in both the samples after *Shodhana*, whereas increase in ether soluble extractive was observed in both the samples. **Table 2** shows data related to different extractive values of two *Dhattura* samples before and after *Shodhana*. Qualitative analysis for various functional groups of all the four samples have shown presence of alkaloids, proteins, carbohydrates, phenols and fixed oils and absence of glycosides, anthrocyanins, saponins, resins, and terpenoids. Phyto-constituents like sugars, phenols, flavonoids, glycosides, tannins, alkaloids etc. are expected to be extracted in the polar solvents. The observed non-appearance of some of the constituents may be due to transfer of these components to the media especially *Gomutra*, which is polar in nature. However *Shodhana* procedure did not alter the qualitative tests for various functional groups and is revealed by presence of alkaloids, proteins, carbohydrates, phenols and fixed oils.

TLC finger printing of raw seed samples showed five spots each with variable R_f values. However after *Shodhana*, only two spots were observed in *Shodhita D.metel* sample and three spots in *D.innoxia* sample. Many previous studies have shown that *Dhattura* seeds contain 0.50 per cent of scopolamine, hyoscyamine and atropine alkaloids¹⁷. Also, Kundu *et al.*, (1991) have reported new alkaloid having related structure of hyoscyamine and hyoscyamine ($C_{20}H_{17}O_2N$)¹⁸. The reduction in number of spots in TLC study indicates that *Shodhana* process may have removed some of the phyto constituents especially alkaloids. This is also evidenced by total alkaloid estimation in which almost 70% reduction in total alkaloid content of both *D.metel* and *D.innoxia* seed samples was observed after *Shodhana*. R_f values of different samples are depicted in **Table 3**. Total alkaloid contents of the seed samples are shown in **Graph 1**.

After *Shodhana*, *D.metel* sample showed 70% increase in total protein content where as *D.innoxia* sample showed an increase of about 42%. The data related to total protein content of seed samples has been shown in **Graph 2**. pH of *Datura metel* was increased by 11% after *Shodhana* whereas it decreased by 11% in *Datura innoxia* sample as depicted in **Graph 3**. The difference in pH value may be due to the fact that the seeds are of two different species.

Further GC-MS analysis has shown scopolamine percentage of *D.metel* and *D.innoxia* to be nil and about 70 to 90% reduction in hyoscyamine content after *Shodhana*. Graph 4 shows percentage of scopolamine and hyoscyamine in both the seed samples as estimated by GC-MS. Complete removal of scopolamine and partial removal of hyoscyamine reflects the importance of *Shodhana* of poisonous plant drugs by means of which one can lessen the toxic effects.

CONCLUSION

The present study shows that *Shodhana* procedure lead to reduction in toxic constituents of two species of *Dhattura* and can be adopted prior to its use in therapeutics. Further analytical study of media used for *Shodhana* is required to ascertain analytical profile and pharmaco-clinical study is required to find out whether purified material is safe as well as efficacious.

REFERENCES

1. Ambikadatta Shastri, editor. Rasa Ratna Samuchhaya, Chaukamba Amarabharati Prakashan, Varanasi; 2003. chapter 10, p. 170.

2. Pandey GS, editor. Bhavaprakasha Nighantu, Chaukamba Amarabharati Prakashan, Varanasi; 2006. p. 634.
3. Indradeo Tripathy, editor. Nighantu, editor, Chaukamba Amarabharati Prakashan, Varanasi; 2003. p.300.
4. Vaidya Bapalal.Nighantu Adarsha.Chaukamba Amarabharati Prakashan, Varanasi; 2005. Volume 2, p. 149.
5. Boumba VA, Mitselou A, Vougiouklakis T. Fatal poisoning from ingestion of *Datura stramonium* seeds. *Vet Hum Toxicol* 2004; 46 Suppl 2: 81-82.
6. Pereira CA, Nishioka S, De D. Poisoning by the use of *Datura* leaves in a homemade toothpaste. *J Toxicol Clin Toxicol* 1994; 32 Suppl 3: 329-31.
7. Chang SS, Wu ML, Deng JF. Poisoning by *Datura* leaves used as edible wild vegetables. *Vet Hum Toxicol*. 1999; 41Suppl 4: 242-45.
8. Kovatsis A, Flaskos J, Nikolaidis J, Kotsaki-Kovatsi VP, Papaioannou N, Tsafaris F. Toxicity study of the main alkaloids of *Datura* species in broilers. *Food and Chemical Toxicology* 1993; 31Suppl 11: 841-45.
9. Brahmashankar Shastri, editor. Yogaratnakara, Chaukamba Amarabharati Prakashan, Varanasi, 2007; p. 169.
10. Yadavji Trikamji Acharya, Rasamrutam; Rasayogavijnaneeyam, translated to english by Joshi Damodar, Chaukamba Amarabharati Prakashan, Varanasi; 2003. p. 284.
11. Baxi AJ, Shukla VJ, Bhatt UB. Methods of Qualitative Testing of some Ayurvedic Formulation, Gujarat Ayurved University, Jamnagar; 2001.
12. Raghuramulu N, Madhavan K, Kalyansundran S. A Manual of Laboratory Techniques, 2nd edition. Hyderabad: National Institute of Nutrition; 2003. p. 36.
13. Anonymous. Ayurvedic Pharmacopoeia of India. Ministry of Health and Family Welfare, New Delhi; 2008, part II, volume II, appendix 3.
14. Ravishankar S. Text book of Pharmaceutical Analysis. Rx Publications, Tirunelveli; 2001, chapter 14, p.3.
15. Anonymous. Quality Standard of Indian Medicine Plants. Indian Council of Medical Research, New Delhi; 2008, Vol-6, p. 110-123.
16. Jeffery GH, Bassett J, Mendham J, Denney RC, editors. Vogel's text book of quantitative chemical analysis. 5th edition. Longman scientific and technical publishers:New York;1989. p. 235.
17. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants (Including the Supplement). Council of Scientific and Industrial Research, New Delhi; 1986, p. 31.
18. Kundu D, Sarkar AK. Identification of a new alkaloid in the seed of *Datura metel* Linn. *Indian J Pharm* 1991; 23: 177-78.

Table 1: Physico-chemical parameters

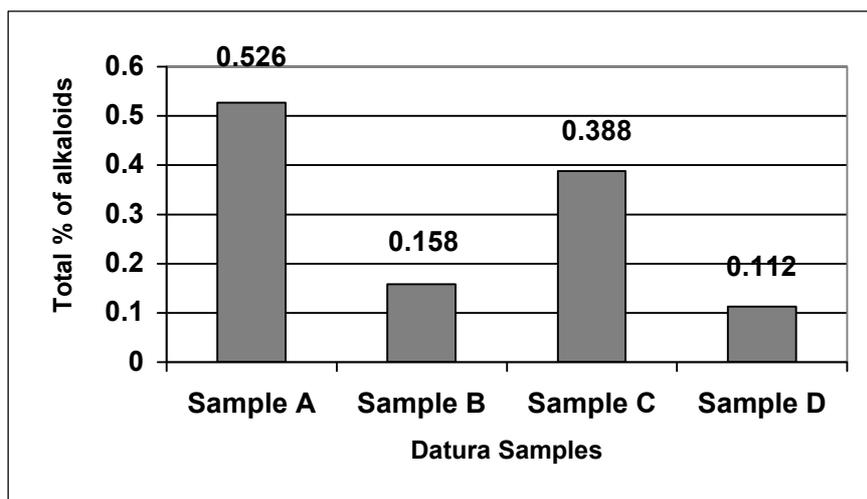
Parameters	Sample A	Sample B	Sample C	Sample D
Loss on drying at 105°C (%w/w)	11.66	8.8	10.18	14.68
Ash value at 500°C (% w/w)	3.7	5.46	4.93	4.40
Acid insoluble ash at 500°C (% w/w)	0.065	0.15	0.065	0.15

Table 2: Extractive values

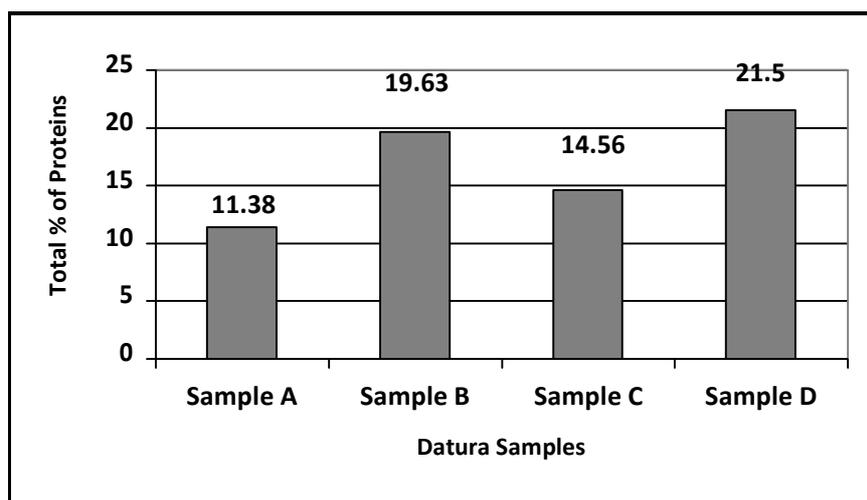
Parameters	Sample A	Sample B	Sample C	Sample D
Water soluble extractive (% w/w)	8.85	5.45	9.3	7.98
Alcohol soluble extractive (% w/w)	20.53	5.4	9.12	7.65
Ether soluble extractive (% w/w)	9.36	18.99	33.06	34.90

Table 3: R_f values of TLC (Methanolic extract of sample – visualization under iodine chamber)

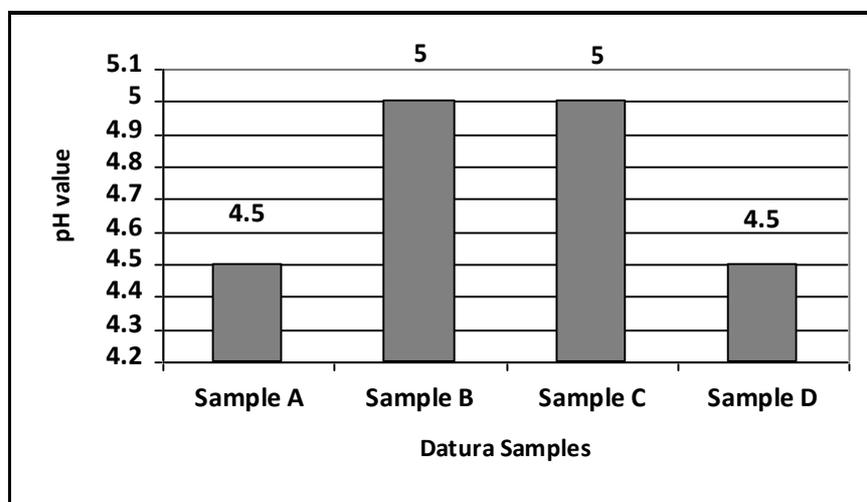
Samples	No. of spots	R _f values
Sample A	5	0.03, 0.19, 0.33, 0.62, 0.94.
Sample B	2	0.28, 0.92.
Sample C	5	0.05, 0.22, 0.37, 0.83, 0.92.
Sample D	3	0.55, 0.74, 0.83.



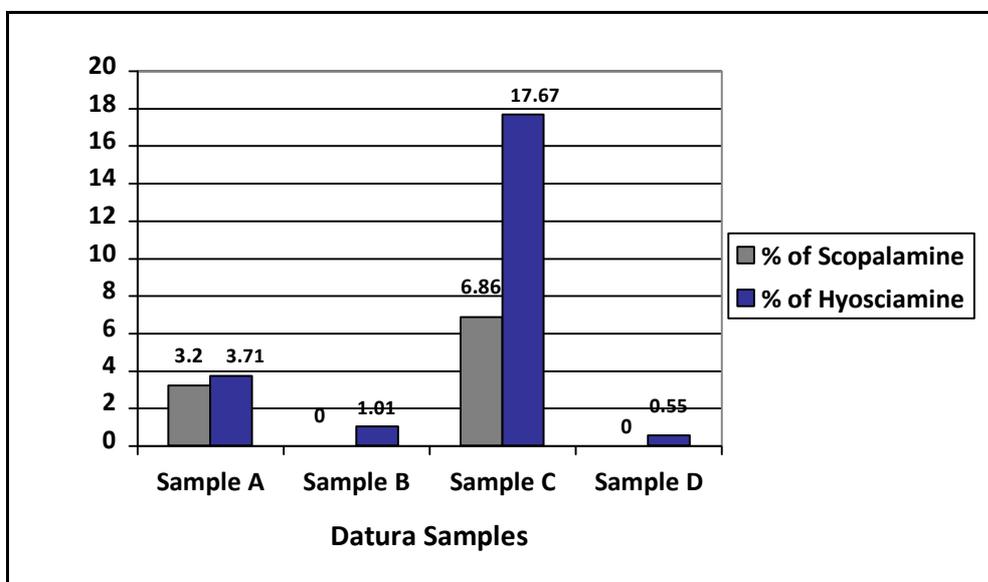
Graph 1: Total alkaloid contents



Graph 2: Total protein content



Graph 3: pH values



Graph 4: GC-MS profile

Source of support: Nil, Conflict of interest: None Declared